ELSEVIER

Pharmacology Biochemistry and Behavior, Vol. 54, No. 2, pp. 479–483, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02277-5

Development of an Affordable Hi-Resolution Activity Monitor System for Laboratory Animals

MARTIN H. TEICHER,[1] SUSAN L. ANDERSEN, PAUL WALLACE, DIANE A. KLEIN AND JACK HOSTETTER

Laboratory of Developmental Psychopharmacology, Mailman Laboratories for Psychiatric Research, McLean Hospital, 115 Mill St., Belmont, MA 02178 USA

Received 2 June 1995; Revised 2 November 1995; Accepted 15 November 1995

TEICHER, M. H., S. L. ANDERSEN, P. WALLACE, D. A. KLEIN AND J. HOSTETTER. *Development of an affordable hi-resolution activity monitor system for laboratory animals.* PHARMACOL BIOCHEM BEHAV **54**(2) 479–483, 1996. – We describe a hardware and software system for recording and analyzing the spatial and temporal pattern of locomotor activity of laboratory animals. The system offers maximal spatial resolution 500-fold greater than existing light beam monitors. An infrared motion analysis systems (MacReflex, Qualysis) simultaneously tracks the location of up to 20 subjects (identified by reflective markers) to within 0.04 mm at a rate of up to 50 Hz. Macintosh software provides measures of distance traveled, amount of area traversed, number of position changes (microevents), average time between movements, number of left and right turns, number of forward movements and reversals, as well as temporal and spatial scaling exponents. This system was validated by comparing these parameters to direct observer scoring of video tapes and other commercially available activity monitors. Our findings show that applying reflective markers to the subjects does not significantly alter activity levels. The effect of pharmacological manipulation with *d*-amphetamine is provided to show the value of the different activity parameters. The main advantages of this system are very high spatial resolution, capacity to monitoring up to 20 animals simultaneously at reasonable cost, and lack of sensitivity of the system to ambient lighting. The main limitation is the need to apply reflective markers.

Activity Amphetamine Behavior Infrared Monitor Spatial scaling Temporal scaling

EARLY activity monitors included tambor cages, stabilimeters, and running wheels in which the animal jiggled, tilted, or rotated the cage to activate mechanical counters (6). Advances in technology produced more sensitive monitors that could detect subtle vibrations (14), perturbations in radiofrequency fields, or disruption of visible or infrared photobeams (8). In general, these devices have produced a single overall index of activity. Microprocessor technology has made it possible to develop activity monitors using photobeam arrays or video cameras that could effectively trace and record the animal's path of movement and have substantially enhanced the quantity and quality of experimental data. To date, the best activity monitors have had a spatial resolution of about 2.5 cm [e.g., (2,5,13)], which, while impressive, ignores a whole range of subtle movements discernable by human observers. Cost has also been a prohibitive factor, especially when it is important to simultaneously monitor many animals.

In the present report we describe the development of an infrared motion analysis system than can simultaneously monitor up to 20 animals (in separate cages) at a sampling rate of up to 50 Hz with a spatial resolution of 0.04 mm. The video camera and computer interface are commercially available (Qualysis, Glasbury, CT), and provide the basis for an extremely precise, economical, high resolution activity monitor. Software to use this instrument, and to simultaneously monitor 12 individually housed rats, has been written by Dr. Teicher for Macintosh computers, and includes an array of interesting measures of locomotor activity.

[1] To whom requests for reprints should be addressed.

INSTRUMENTATION

Components of the System

The hardware for the activity monitor consists of two principle components, a high resolution CCD infrared video camera with a 50 Hz infrared strobe, and an f8.5 lens with 39° viewing angle (MacReflex Camera Model NP), and a videoprocessor (Model VP-I; Qualysis, Glasbury, CT). The videoprocessor provides hardware analysis of the video camera signal and outputs processed data on marker position through an RS232C or RS422 port (maximum baud rate of 500 K). The videoprocessor is interfaced to a Macintosh computer that stores the data in real time. The system is specifically designed to detect and record the precise vertical and horizontal position of small, light-weight infrared reflectors. The device has a spatial resolution of 1 : 30,000, and it divides its field of view into approximately 20,000 vertical and 27,000 horizontal units. For a 1.2 × 0.9 m field of view, the centerpoint of each marker will be located in a Cartesian coordinate system with a spatial resolution of 40 μm, and as many as 20 markers can be simultaneously tracked up to 50 times per second. This is a very high precision instrument with a measurement error of $<0.001\%$. A simple software program establishes the duration of the recording period and stores to hard disk (in random access compressed binary format), the frame number, the vertical and horizontal (X-Y) position of the centerpoint of each marker, and the total area of each marker (in pixels). Data is stored in real time, so that an interruption in power does not cause loss of previously collected data. The markers can also be viewed simultaneously in real time with a video monitor.

A second program is then utilized to view the results at high speed, and to separate out results from each subject based on the cage location. Individual subject measures (X-Y coordinates and marker size for each reading) are stored in a separate ASCII text files.

Data Analysis

Individual subject files are analyzed using an activity analysis program that can be run in batch processing mode. Activity data are analyzed based on a powerful schema developed by Paulus and Geyer (9–11) for the study of X-Y time series data. The fundamental unit of analysis is the microevent, which is defined in three dimensions by its position and duration. A new microevent begins when the centerpoint of the marker moves more than a predefined distance (nominally 4 mm) from the last microevent. Duration is the amount of time spent at this location before the subject moves to the next microevent position. The complete sequence of microevent positions is graphed and analyzed to determine total displacement (in pixels and mm), number of position changes, and the average amount of time between position changes. By examing microevent positions in sequences of three movements, the software calculates the number of right turns (angles of 90 ± 60°), left turns (270 ± 60°), forward movements (0 ± 30°), and reversals (180 ± 30°).

The major feature of this approach is the derivation of temporal and spatial scaling exponents, which have been used to provide insight into the behavior of complex systems (9–11). The spatial scaling exponent d, is a measure of the complexity of the movement path, and is calculated by ascertaining the rate of information decay at progressively lower levels of temporal resolution (10). It corresponds to the concept of fractal dimensions, and ranges from 1.0 (straight line movement) to 2.0 (hypercomplex, convoluted movement patterns). The temporal scaling exponent, is calculated from the stochastic log-log reciprocal relationship between the frequency of occurrence of microevents of different durations. [Mathematically, it can be shown that shorter duration microevents occur more frequently than longer duration microevents, and the relationship between microevent duration and frequency of occurrence is well defined by a linear regression after log transformation; (10)]. This parameter varies between 0 (inactivity) and 1 (incessant activity), and indicates the degree to which a subject is "acting in its environment" (10).

VALIDATION OF THE ACTIVITY MONITOR

Effects of Marking the Animals

The greatest limitation of the infrared motion analysis system is that animals need to be marked with an infrared reflector. We have found that a moderately large square (1 × 1 cm) of Scotch 3M reflective tape, glued to the animal's anterior dorsal surface (immediately posterior to the neck) with cyanoacrylate (after shaving the region), provides an excellent reflector. The relatively large size of the marker ensures that it will remain on during the test period, and that the marker will be detected regardless of the position the animal assumes. In the present study we sought to ascertain whether the marker exerted a significant effect on the animal's pattern of activity. Twelve male rats, 60 days of age, were tested. Half of the group had reflective markers applied the evening before testing and were returned to the colony room with unlimited access to food and water. The next day subjects were then placed on a Stoelting Electronic Activity Monitor, which records activity counts as perturbation in a radio frequency field, and activity counts were obtained at 5-min intervals during a 60-min test session. As seen in Fig. 1, marking the animal had relatively little effect. There was no significant effect of marking on mean activity, $F(1, 10) = 0.04, p > 0.8$, nor was there a significant interaction between marking and time on activity, $F(11, 110) = 0.65, p > 0.7$.

Videotape Validation

In order to validate overall activity levels (displacement) generated by the software, subjects were observed following a systemic injection of either saline ($n = 12$) or 20 mg/kg SC fluoxetine ($n = 12$). Animals were then videotaped while monitored simultaneously with the infrared camera. An observer who was blind to the conditions of the subjects used a time-sampling method, in which the animals were identified

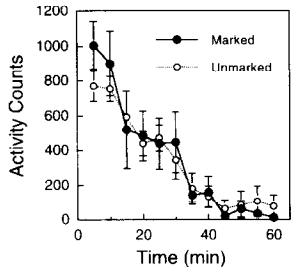

FIG. 1. Effects of marking with reflective tape on levels of activity of adult rats habituating to a novel environment for 60 min. Data was obtained from a Stoelting activity monitor. Application of reflective tape the night before exerted no significant effect on levels of activity or rate of habituation.

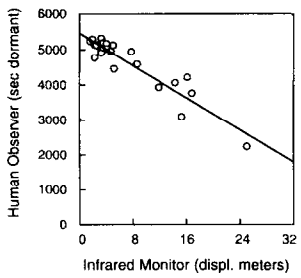

FIG. 2. Correlation between the infrared motion analysis system calculating distance traveled (displacement) and human scoring from videotapes of seconds spent dormant. Subjects were studied for 60 min.

as active or dormant every 60 s. There was a very strong negative linear relation between displacement and seconds spent dormant ($r = -0.933$, $p < 0.0001$; Fig. 2), and between microevents and seconds dormant ($r = -0.921$, $p < 0.0001$).

Comparison with Stoelting Activity Monitor

For a further test of validation we compared the rate at which animals would habituate to a novel environment as assessed by the infrared motion analysis system and a commercial animal activity monitor. Twenty-two adult male rats were studied. Eleven subjects were tested on a Stoelting Electronic Activity Monitor and 11 male rats were tested on the Infrared Motion Analysis System as described. Subjects were identified with reflective markers (applied the night before testing) and activity was recorded for the first 60 min after they were placed into an unfamiliar environment (standard opaque plastic rat breeding cages measuring 46 × 26 × 20 cm) on each device to assess the rate of habituation in activity over time. As shown in Fig. 3, the pattern and rate of habituation in activity were virtually identical across the two different devices, $F(11, 220) = 0.403$, $p = 0.95$).

Effects of d-*Amphetamine on Spontaneous Activity*

The capacity of the infrared motion analysis system to detect drug effects was ascertained by challenging rats with moderate and high doses of *d*-amphetamine. A great deal of previous research has shown that moderate doses of *d*-amphetamine (1.0 mg/kg or less) markedly increase locomotion, while higher doses produce a stereotypic and preseverative movement pattern (7). As a consequence, rats tend to ambulate more after moderate doses than high doses. Adult male rats (350–400 g) had reflective markers applied and were habituated overnight to the test chamber with access to food and water. At the time of testing, the food and water were removed and a nonreflective wire mesh screen covered the cages. Drugs were administered in doses of 0, 0.3, 1.0, and 5.0 mg/kg *d*-amphetamine sulfate by intraperitoneal injection ($n = 12$, 6, 6, and 9, respectively). Activity was recorded with the infrared system for 60 min, commencing 1 h after drug injection.

As shown in Fig. 4, amphetamine induced a marked effect on the pattern of motor activity. Moderate doses substantially increased the amount of general movement throughout the cage. At the highest dose activity was usually restricted to a more limited portion of the cage. *d*-Amphetamine markedly increased the number of position changes [microevents: $F(3, 29) = 6.81$, $p = 0.001$], and number of forward, $F(3, 29) = 4.75$, $p = 0.008$, and reverse $F(3, 29) = 14.14$, $p < 0.00001$, movements (Fig. 5). Interestingly, the ratio of forward movement to reversals was nearly equal when animals received vehicle injections (0.76 ± 0.08). At moderate substereotypic doses this ratio markedly increased (e.g., 2.32 ± 0.41 with 1.0 mg/kg), indicating a predilection for forward motion over reversals. However, this ratio substantially declined at the highest dose [0.95 ± 0.39; $F(3, 29) = 5.39$, $p = 0.005$]. High ratios of forward-to-reverse movement indicate vigorous locomotion, while lower ratios reflect hesitant exploration or stereotypy. Moderate doses of *d*-amphetamine also caused animals to move throughout a far greater area of their cages, $F(3, 29) = 11.90$, $p < 0.00003$. This parameter declined significantly between 1.0 and 5.0 mg doses, $t(13) = 3.72$, $p = 0.003$, indicating that stereotypic doses cause animals to perseverate in a limited region of the cage. The time spent immobile also declined markedly with amphetamine, $F(3, 29) = 6.46$, $p < 0.002$, indicating a substantial effect of amphetamine on arousal at all doses.

Figure 6 shows that amphetamine exerted prominent effects on the spatial and temporal complexity of activity, $F(3, 29) = 7.44$, $p < 0.0008$; and $F(3, 29) = 4.8$, $p < 0.008$, respectively. The spatial scaling exponent decreased at moderate doses of amphetamine, suggesting a more linear path of movement, consistent with vigorous ambulation. At the high dose

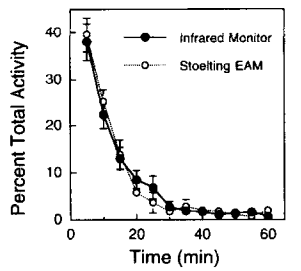

FIG. 3. Rate of habituation of activity of adult rats placed in a novel environment as assessed using the infrared motion analysis system and Stoelting electronic activity monitors. Both devices indicated very comparable rate and pattern of habituation of activity. Total activity was derived from displacements with the infrared system and activity counts were obtained from the Stoelting monitor.

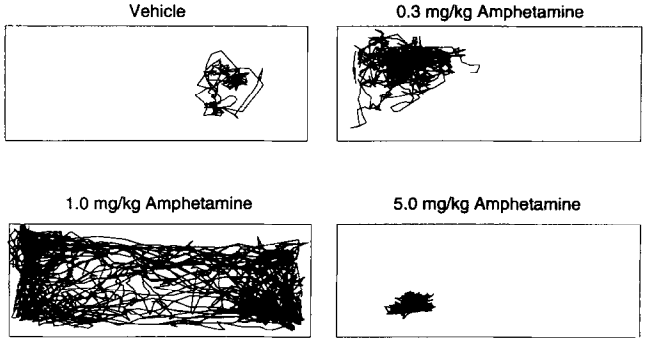

FIG. 4. A composite of typical patterns of activity of adult rats treated with vehicle, 0.3, 1.0, or 5.0 mg/kg, IP of *d*-amphetamine. Peak activity for minutes 60–120 is depicted here.

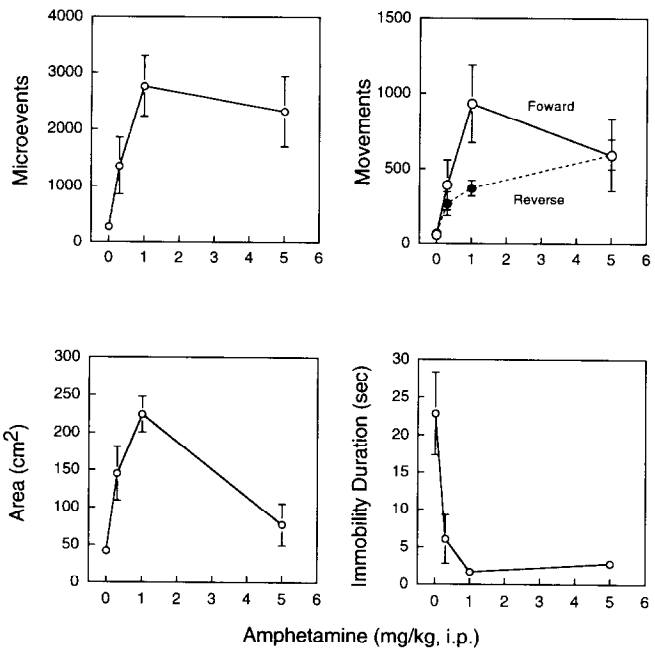

FIG. 5. Amphetamine (vehicle, 0.3, 1.0, or 5.0 mg/kg, IP) exerted a dose-dependent effect on the number of microevents, movements, area, and immobility duration as detected by infrared motion analysis. Means were obtained from a 60-min interval of peak activity.

of amphetamine the spatial scaling exponent dramatically increased [1.0 vs. 5.0 mg/kg; $t(13) = 3.56$, $p = 0.003$], indicating a hypercomplex movement pattern, consistent with stereotypy. The temporal scaling exponent was increased substantially by all doses of amphetamine, and there were no significant differences between 0.3, 1.0, and 5.0 mg/kg, indicating that amphetamine had a robust activating effect throughout this dosage range. Thus, measures derived from the infrared motion analysis system, can differentiate overall activating effects from more specific effects on the pattern and complexity of movement.

DISCUSSION

We have introduced an affordable, objective means of quantifying locomotor activity. This system offers many advantages over other automated activity monitors. First, several subjects can be analyzed simultaneously using a single camera and computer to assure comparability of the data. Second, the device provides far greater spatial resolution than commercial devices. The present device provides excellent temporal resolution (50 Hz) at moderate expense, and even greater temporal resolution (100 Hz) can be obtained for additional money. Further, through the use of microevents and scaling exponents, important data on drug effects can be readily obtained (3). In the present report we demonstrate the capacity of the device to distinguish between the sterotypic and arousing properties of *d*-amphetamine. We have also applied this technology to study the effects of fluoxetine (15), which in previous studies was found to exert no effect on activity (1,12). Using the infrared motion analysis system we found that fluoxetine exerted no significant effect on locomotion. However, fluoxetine (10-30 mg/kg) significantly affected temporal scaling and immobility duration, indicative of an activating effect and production of restlessness but not stereotypy (15). Lower doses exerted no significant effects (15), confirming that the monitor does not produce artifactual false-positive results.

Finally, we have also found the device to be extraordinarily flexible and have used the same technology to record the pattern of head, trunk, and elbow movements of children with Attention-Deficit Hyperactivity Disorder (ADHD) and normal controls, while seated in front of a computer administering a continuous performance task. The spatial complexity of the movement pattern readily distinguished children with ADHD from controls (16).

In the present study we essentially limited spatial sensitivity to 4 mm to exclude very fine movements such as shivers, tremors, twitches, and myoclonic jerks. The device however is capable of 100-fold greater resolution, which may be useful in other studies and may enable the device to detect subtle differences in activity undetectable by a human observer. Another key advantage of the device is that the infrared technology facilitates monitoring of rats in the dark or throughout their light-dark cycle. Theoretically, it should be possible to apply pattern recognition algorithms to the data to identify specific categories of behaviors (5). However, accurate pattern recognition may require three-dimensional information (5), which can either be derived from the size of the marker or through the use of a second camera and interface.

The primary limitation of the system is the need to use reflective markers. These have reliably remained in place for up to 7 days to study activity rhythms, and they can be removed and reapplied for repeated measure studies. Mechanically, they have not presented a significant problem using dorsal SC injections (15). Blood level studies that we have conducted to date do not indicate any significant interference with absorption of subcutaenous fluoxetine. However, other drugs have not been studied. The device provides X-Y measures, but does not directly provide information about rearing or other Z-plane movements. This can be extracted from changes in the size (total area) of the marker. A complete 3D system is also commercially available that uses two cameras. However, it may prove difficult to appropriately mark several animals for simultaneously 3D study.

Overall, we have found this to be an accurate, reliable, and flexible activity monitoring system that is particularly well suited for the detailed quantitative study of drug effects.

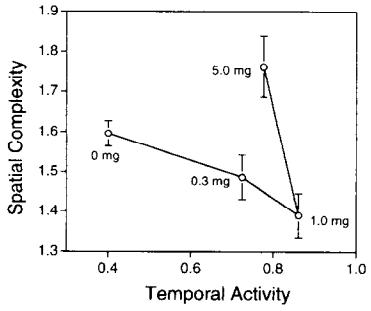

FIG. 6. Spatial and temporal scaling exponents for subjects treated with vehicle, 0.3, 1.0, or 5.0 mg/kg of *d*-amphetamine over a 60-min period. Note the dramatic rise in spatial scaling with little change in the temporal domain at 5.0 mg/kg.

REFERENCES

1. Callaway, C. W.; Johnson, M. P.; Gold, L. H.; Nichols, D. E.; Geyer, M. A. Amphetamine derivatives induce locomotor hyperactivity by acting as indirect serotonin agonists. Psychopharmacology (Berlin) 104:293–301; 1991.
2. Forster, M. J. Age differences in locomotor behavior of C57BL/6Nia Mice: A data management approach. Pharmacol. Biochem. Behav. 27:545–551; 1987.
3. Geyer, M. A.; Russo, P. V.; Masten, V. L. Multivariate assessment of locomotor behavior: Pharmacological and behavioral analyses. Pharmacol. Biochem. Behav. 25:277–288; 1986.
4. Hopper, D. L.; Kernan, W. J.; Wright, J. R. Computer pattern recognition: An automated method for evaluating motor activity and testing for neurotoxicity. Neurotox. Teratol. 12:419–428; 1990.
5. Kernan, W. J.; Mullenix, P. J.; Hopper, D. L. Pattern recognition of rat behavior. Pharmacol. Biochem. Behav. 27:559–564; 1987.
6. Kinnard, W. J.; Watzman, N. Techniques utilized in the evaluations of psychotropic drugs on animal activity. J. Pharm. Sci. 55: 995–1012; 1966.
7. Moore, K. E. Amphetamines: Biochemical and behavioral actions in animals. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. Handbook of psychopharmacology: Stimulants. Vol. 11. 1976: 41–98.
8. Moross, G. G.; Kaufman, G. I. Activity monitor for small animals. Physiol. Behav. 16:493–495; 1976.
9. Paulus, M. P.; Geyer, M. A. A scaling approach to find order parameters quantifying the effects of dopamine agents on unconditioned motor activity in rats. Prog. Neuropsychopharmacol. Biol. Psychiatry 15:903–919; 1991.
10. Paulus, M. P.; Geyer, M. A. The effects of MDMA and other methylenedioxy-substituted phenyalkylamines on the structure of rat locomotor activity. Neuropsychopharmacology 7:15–31; 1992.
11. Paulus, M. P.; Geyer, M. A. Three independent factors characterize spontaneous rat motor activity. Behav. Brain Res. 53:11–20; 1993.
12. Rempel, N. L.; Callaway, C. W.; Geyer, M. A. Serotonin$_{1B}$ receptor activation mimics behavioral effects of presynaptic serotonin release. Neuropsychopharmacology 8:201–211; 1993.
13. Sanberg, P. R.; Zoloty, S. A.; Willis, R.; Ticharich, C. D.; Rhoads, K.; Nagy, R. P.; Mitchell, S. G.; LaForest, A. R.; Jenks, J. A.; Harkabus, L. J.; Gurson, D. B.; Finnefrock, J. A.; Bednarik, E. J. Digiscan activity: Automated measurement of thigmotactic and stereotypic behavior in rats. Pharmacol. Biochem. Behav. 27:569–572; 1987.
14. Teicher, M. H.; Green, W. T. A digital readout vibrational activity monitor for newborn animals. Physiol. Behav. 18:747–750; 1977.
15. Teicher, M. H.; Klein, D. A.; Andersen, S. L.; Wallace, P. Development of an animal model of fluoxetine akathisia. Prog. Neuropsychopharmacol. Biol. Psychiatry (in press).
16. Teicher, M. H.; Ito, Y.; Glod, C. A.; Barber, N. I. Objective measurement of hyperactivity and attentional problems in ADHD. J. Am. Acad. Child Adol. Psychiatry (in press).